PCT/EP03/12191

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

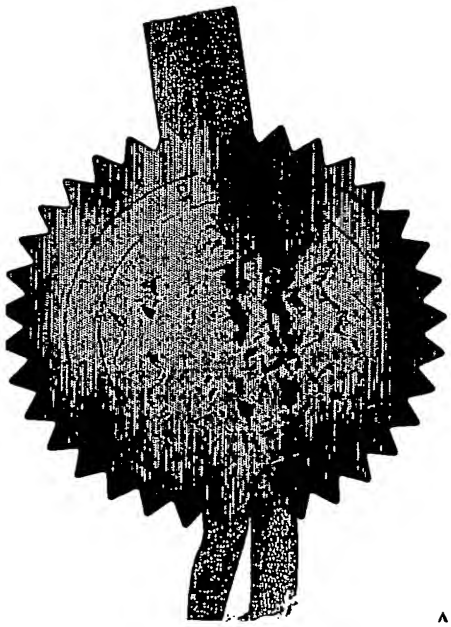
PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.



Signed Andrew Cerny

Dated 15 October 2003

PCT/EP 0 3 / 1 2 1 9 1

25JUL03 E825211-3 D02029
P01/7700 0.00-0317381.2

Patents Form 1/77

Patents Act 1977
(Rule 16)

The Patent Office

THE PATENT OFFICE
H
24 JUL 2003
LONDON

1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office
Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference — CJ/KMM/VB60409P

2. Patent application number
(The Patent Office will fill in his part) — 0317381.2

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

GlaxoSmithKline Biologicals s.a.
Rue de l'Institut 89, B-1330 Rixensart, , Belgium

Patents ADP number *(if you know it)*

810127001

If the applicant is a corporate body, give the country/state of its incorporation

Belgian

4. Title of the invention — Drying Method

5. Name of your agent *(if you have one)*

Corporate Intellectual Property

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

GlaxoSmithKline
Corporate Intellectual Property (CN9 25.1)
980 Great West Road
BRENTFORD
Middlesex TW8 9GS

Patents ADP number *(if you know it)*

8072555006

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right

Drying Process

The present invention relates to the preservation of biological and other labile samples, to such preserved samples and to a novel process for preserving such samples. The process involves drying the labile sample within a container with a water repellent interior surface, for instance a siliconised container, in the presence of a stabilising agent such as a polyol. The novel process comprises adding a sample including an active agent and a stabilising agent to a water-repellent container, subjecting the sample to such temperature and pressure conditions to cause the sample to bubble and form a foam. After formation of the foam, pressure and temperature conditions are maintained or adjusted so that solvent is removed and the foam dries to form a solid. Further provided by the present invention are compositions preserved by the process of the present invention and in particular preserved vaccine compositions. Another aspect of the invention is a container with a water repellent internal surface, holding a foamed glass comprising an active agent.

There is a need to extend the stability and thus the shelf life of labile samples, particularly biological samples. Traditionally, this has been accomplished using the process of freeze drying in which a solution of the substance is made and the sample is frozen. During the primary drying phase, most of the water is removed by sublimation from ice under reduced pressure conditions and a porous 'cake' is formed. This is usually followed by a secondary drying phase when the pressure and temperature are changed and water is evaporated from the solid 'cake'. The resulting lyophilised sample has improved stability compared to a liquid formulation. However, the freeze drying process is lengthy, expensive and can be the rate limiting step in a production process.

Product variability is also a problem when many samples are being batch lyophilised in a large dryer unit. The conditions on the shelves of the freeze dryer vary between different positions leading to samples lyophilising at different rates under different conditions. For certain biological materials such as live virus, there can be significant loss of activity during the freeze drying process (Pikal (1994) ACS Symposium 567:

120-133). Many freeze dried substances are still unstable at ambient temperature (Carpenter et al (1994) ACS Symposium 567; 134-147).

Damage caused by the process of freezing may be circumvented to some degree by the use of stabilising agents such as polyols. Further improvements on the process of lyophilisation have also been made by avoiding freezing the sample during the process and removing water by boiling (WO96/40077; US6306345). This method involves preparing a mixture of a glass-matrix forming material in a suitable solvent together with the sample to be preserved, evaporating bulk solvent from the mixture to obtain a syrup, exposing the syrup to a pressure and temperature sufficient to cause boiling of the syrup and removing residual solvent.

A similar method was described in US5,766,520, in which the process involves partially removing the water to form a viscous fluid and further subjecting the syrup to vacuum to cause it to 'boil' and further drying at temperatures substantially lower than 100 °C. This method still suffers from some of the problems of conventional freeze-drying. When the process is carried out in a large freeze-dryer, samples will dry at different rates depending on their position on the shelf and this leads to different samples loosing different amount of activity during the drying process. This leads to a lack of consistency within a batch.

Trehalose is a polyol that is favoured for its stabilising properties. Trehalose is a naturally occurring, inert, non-reducing and non-toxic, glass-forming disaccharide that was initially found to be associated with the prevention of desiccation damage in some plants and animals. Trehalose is useful in preventing denaturation of a wide variety of substances including proteins, viruses and foodstuffs during desiccation and subsequent storage partly because it has a relatively high glass transition temperature (ca 120 °C in the anhydrous state) (US4891319; US5149653; US5026566). Trehalose also stabilises enzymes (Argall and Smith (1993) Biochem. Mol. Biol. Int. 30; 491). Trehalose and a wide variety of stabilising polyols have also been found to be useful in improving the preservation of freeze-dried samples, especially in cases where the

sample is prone to loss of activity during the freeze-drying process. Other sugars useful in lyophilisation techniques include sucrose and lactose.

The present invention relates to an improved method of preserving an active agent comprising the steps of preparing a preservation sample by suspending or dissolving the active agent in a solution of a stabilising agent; inserting the preservation sample into a container with an internal water repellent surface; subjecting the preservation sample to such temperature and pressure conditions that the preservation sample forms a foam; and removing solvent until the foam dries to form a solid.

The use of a container whose internal surface is water repellent, for instance a siliconised vial, in the process of the invention has several advantages over previously described methods of preserving samples as a foamed glass. The presence of a water repellent internal surface of the container, affects the formation of bubbles within the solution. This is evidenced by the greater ease of degassing the preservation sample by reducing the pressure and also the increased reproducibility of foam formation when using a container with water repellent internal surfaces.

The result of more efficient foam formation in vials with a water repellent internal surface, is that a lower concentration of polyol can be used in the preservation sample. This in turn leads to a shorter time in a freeze dryer being required to dry the sample. Since batch lyophilisation is often the rate limiting step of industrial processes, a reduction is the time taken to dry a sample can allow increased production capacity.

Further advantages of using containers with a water repellent inner surface, arise if the active agent to be preserved is a biological material, such as a protein polysaccharide, or nucleic acid as found in vaccines or other medicines. Such molecules dried in a container with a water repellent inner surface, using the process of the invention contain a smaller amount of stabilising polyol and are therefore even easier and quicker to re-dissolve just prior to injection. This decreases the amount of time required to give the inoculation. A second advantage is the ease of removing the

reconstituted vaccine when the container surface repels water. This results in more efficient and effective delivery of the vaccine.

It is particularly advantageous to dry IPV (inactivated polio virus, the active ingredient in injectable polio vaccine) using the process of the invention. IPV is present in prior vaccines as a liquid formulation (WO99/48525). Problems have arisen on attempting to use a solid formulation of IPV in a vaccine since standard freeze drying procedures lead to a loss of IPV antigenicity. The process of the invention leads to much higher integrity of the polio virus antigens, due partially to the reduced time required by the process of the invention. It is advantageous to formulate IPV with together with Hib, preferably as a solid formulation, because the presence of IPV can reduce the amount of interference associated with combining Hib with other antigens.

The process of the invention is advantageous over normal freeze drying since the running cycle is shorter and is and requires less refrigeration making it more energy efficient. Since the drying process is often the rate limiting step of a process, the use of the method of the invention leads to higher levels of production at reduced expense. The foam dried solid that is achieved as the product of the process is also easier to reconstitute than a standard freeze dried cake, due to its greater surface area.

Description of figures

Figure 1 – Photographs of vials containing the preservation sample at different stages of the foam drying process.

A - Shows the appearance of the preservation samples as inserted into the freeze drying as a liquid formulation.

B - Shows the appearance of the preservation samples as the pressure is reduced to 1.5mbars. The samples begin to freeze at slightly different rates due to differing conditions in each vial.

C - Shows the appearance of the preservation samples at 0.1mbars, where all samples have become completely frozen.

D - Shows the appearance of the preservation samples as the pressure is increased to 0.8 – 3.5mbars. A foamed glass is formed as the preservation sample foams and solvent evaporates.

Detailed description

The method of the invention is used for preserving an active agent and comprises the steps of:

- preparing a preservation sample by suspending or dissolving an active agent in a solution of a stabilising agent;
- inserting the preservation sample into a container with a water repellent interior surface;
- subjecting the preservation sample to such temperature and pressure conditions that the preservation sample forms a foam; and
- removing solvent until the foam dries to form a solid.

The method is useful for extending the shelf life of labile products which rapidly loose activity when stored in solution. It is particularly applicable for use where a lower concentration of the glass forming polyol is advantageous and a shorter drying process is preferred.

A dried solid formulation is a formulation which has had solvent removed by a process of lyophilisation, sublimation, evaporation or desiccation so that less than or equal to 20%, 15%, 10%, 7%, 5%, 4%, preferably 3%, 2% or most preferably 1% solvent remains. The term 'solid' comprises glasses, rubbers or crystalline solids with a solid appearance. Any of the methods described above can be used to make such a dried solid. Solvent is removed by sublimation, boiling or evaporation, preferably by evaporation.

<u>Preparation of the preservation sample</u>

Any stabilising agent is suitable for use in the first step of this invention. Suitable materials include, but are not limited to, all polyols, including carbohydrate and non-carbohydrate polyols. Preferably the stabilising polyol enables the active agent to be stored without substantial loss of activity by denaturation, aggregation or other means. Particularly suitable materials include sugars, sugar alcohols and carbohydrate derivatives. Preferably, the glass forming polyol is a carbohydrate or derivatives thereof, including glucose, maltulose, iso-maltulose, lactulose, sucrose, maltose, lactose, iso-maltose, maltitol, lactitol, palatinit, trehalose, raffinose, stachyose, melezitose or dextran, most preferably trehalose, sucrose, sorbitol, raffinose, mannitol, lactose, lactitol or palatinit, most preferably sucrose, sorbitol, lactose or trehalose.

Bacterial polysaccharides are particularly advantageous for use as a stabilising agent in an immunogenic composition since they can act both as a stabilising agent and an immunogen.

Carbohydrates include, but are not limited to, monosaccharides, disaccharides, trisaccharides, oligosaccharides and their corresponding sugar alcohols, polyhydroxyl compounds such as carbohydrate derivatives and chemically modified carbohydrates, hydroxyethyl starch and sugar copolymers. Both natural and synthetic carbohydrates are suitable for use. Synthetic carbohydrates include, but are not limited to, those which have the glycosidic bond replaced by a thiol or carbon bond. Both D and L forms of the carbohydrates may be used. The carbohydrate may be non-reducing or reducing. Where a reducing carbohydrate is used, the addition of inhibitors of the Maillard reaction is preferred.

Reducing carbohydrates suitable for use in the invention are those known in the art and include, but are not limited to, glucose, maltose, lactose, fructose, galactoase, mannose, maltulose and lactulose. Non-reducing carbohydrates include, but are not limited to, non-reducing glycosides of polyhydroxyl compounds selected from sugar alcohols and other straight chain polyalcohols. Other useful carbohydrates include raffinose, stachyose, melezitose, dextran, sucrose, cellibiose, mannobiose and sugar

alcohols. The sugar alcohol glycosides are preferably monoglycosides, in particular the compounds obtained by reduction of disaccharides such as lactose, maltose, lactulose and maltulose.

Particularly preferred carbohydrates are trehalose, sucrose, sorbitol, maltitol, lactitol, palatinit and glucopyranosyl-1→6–mannitol.

Amino acids can act as stabilising agents and can be used by themselves and preferably in combination with a polyol. Preferred amino acids include glycine, alanine, arginine, lysine and glutamine although any amino acid, or a combination of amino acids, peptide, hydrolysed protein or protein such as serum albumin can act as a stabilising agent.

The concentration of the stabilising agent used in the process of the invention may be between 1% and 50% weight/volume, preferably 1-5%, 5-10%, 5-10%, 15-20%, 20-25% or 25-50%, most preferably less than 25%. The amounts of stabilising agent required is proportional to the amount of salts present. Therefore, although levels of stabilising agent between 3% and 10% are preferred, higher concentrations of 10% to 25% may be required to dry samples with a high salt content.

Container

Different mixtures and various container shapes and sizes can be processed simultaneously. Ideally, the container size used is sufficient to contain the initial mixture and accommodate the volume of the solid formed thereof. Typically, this is determined by the mass of the glass forming material, the surface area of the container and the conditions of the foamed glass formation. The mass of glass forming material must be sufficient to give viscous syrup to be foamed which translates practically as a minimal mass per unit area of container surface. This ratio varies from mixture to mixture and container used, but is easily determined empirically by one skilled in the art by following the procedures set forth herein. Any such vials can be used, including Wheaton moulded and tube-cut vials.

The process of the invention uses containers with a water repellent interior surface. This is achieved through coating the interior surface with a hydrophobic composition, for instance by siliconisation. Siliconisation is achieved by processes that are well known to those skilled in the art. In one method, the container is siliconised by rising the interior of the container with an emulsion of silicone, followed by processing through an oven at high temperature, typically 350 °C. Alternatively, the water repellent interior surface is achieved by the container being made of a water repellent composition.

The water repellent interior surface of the container makes foam formation more likely to occur and more reproducible. This allows lower polyol concentrations to be used in the preservation sample which in turn decreases the length of time necessary to dry the sample, reduces the effect of Maillard reactions or other interactions with the polyol harming the active agent. Where the preservation samples comprises a vaccine, the resultant foamed glass is reconstituted quickly due to the lower amount of polyol present and the resultant vaccine solution is less viscous, allowing easier administration.

Although singular forms may be used herein, more than one glass matrix-forming material, more than one additive, and more than one substance may be present. Effective amounts of these components are easily determined by one skilled in the art.

Solvent

The solvent into which the glass matrix-forming material is mixed can be aqueous, organic, or a mixture of both. The use of combinations of organic and aqueous solvents can provide an additional benefit, as the use of a volatile organic solvent enhances the foamed glass formation. Enhanced glass formation can be achieved by using a volatile or decomposing salt as discussed below. Additionally, sufficient aqueous solvent to dissolve the glass matrix-forming material and sufficient organic solvent to dissolve a hydrophobic substance may be used, allowing the formation of foamed glass incorporating hydrophobic substance(s).

The choice of solvent will depend upon the nature of the material chosen for glass matrix formation, as well as the nature of any additive and/or substance to be incorporated. The solvent should be of a nature and of sufficient volume to effect adequate solubilization of the glass matrix-forming material as well as any additive and/or substance. If the substance is a hydrophilic material, the liquid will preferably be aqueous to avoid any potential loss of activity due to deleterious solvent interactions. Preferably, the aqueous solvent includes any suitable aqueous solvent known in the art, including, but not limited to, water and biological buffer solutions. Preferably, the aqueous solvent is present in an amount of 5 to 98% by volume, more preferably 80-98% by volume, most preferably 85-98% by volume.

The volume of solvent can vary and will depend upon the glass matrix-forming material and the substance to be incorporated as well as any additives. The minimum volume required is an amount necessary to solubilise the various components. However, homogeneously dispersed suspensions of the substance(s) can also be used. Suitable amounts of the components in specific embodiments are easily determinable by those skilled in the art in light of the examples provided herein.

Various additives can be put into the glass matrix-forming material. Typically, the additives enhance foam formation and /or the drying process or contribute to the solubilization of the substance. Alternatively, the additives contribute to the stability of the substance incorporated within the solid. One or more additives may be present.

As an example, addition of volatile/effervescent salts allows larger initial volumes and results in higher surface area within the foamed glass, thus effecting superior foam formation and more rapid drying. As used herein, volatile salts are salts which volatilise under the conditions used to produce a foamed glass. Examples of suitable volatile salts include, but are not limited to, ammonium acetate, ammonium bicarbonate and ammonium carbonate. Salts that decompose to give gaseous products also effect enhanced foam formation and more rapid drying. Examples of such salts are sodium bicarbonate and sodium metabisulphite. Preferably, the volatile salts are

present in an amount of from about 0.01 to 5 M. Concentrations of up to 5 M are suitable for use herein. The resultant foamed glass has uniform foam conformation and is significantly drier compared to foamed glass in which volatile/effervescent salts are not used.

Volatile organic solvents can also be used in the initial mixture in order to improve the formation of a foamed glass. Examples of suitable volatile organic solvents include, but are not limited to, alcohols, ethers, oils, liquid hydrocarbons and their derivatives. While the volatile organic solvent may be used as the sole solvent for the glass matrix-forming material and/or substance, they are more commonly used in aqueous/organic mixtures.

Another suitable additive is a foam stabilising agent, which can be used in combination with either the volatile or decomposing salt and/or organic solvent. This may either be a surface active component such as an amphipathic molecule (i.e. such as phospholipids and surfactants) or an agent to increase the viscosity of the foaming syrup, such as a thickening agent such as guar gum and their derivatives.

Another additive is an inhibitor of the Maillard reaction. Preferably, if the substance and/or glass matrix-forming material contains carbonyl and amino, imino or guanidino groups, the compositions further contain at least one physiologically acceptable inhibitor of the Maillard reaction in an amount effective to substantially prevent condensation of amino groups and reactive carbonyl groups in the composition. The inhibitor of the Maillard reaction can be any known in the art. The inhibitor is present in an amount sufficient to prevent, or substantially prevent, condensation of amino groups and reactive carbonyl groups. Typically, the amino groups are present on the substance and the carbonyl groups are present on the glass matrix forming material, or the converse. However, the amino acids and carbonyl groups may be intramolecular within either the substance or the carbohydrate.

Various classes of compounds are known to exhibit an inhibiting effect on the Maillard reaction and hence to be of use in the compositions descried herein. These

compounds are generally either competitive or non-competitive inhibitors of the Maillard reaction. Competitive inhibitors include, but are not limited to, amino acid residues (both D and L), combinations of amino acid residues and peptides. Particularly preferred are lysine, arginine, histidine and tryptophan. Lysine and aarginine are the most effective. There are many known non-competitive inhibitors. These include, but are not limited to, aminoguanidine and derivatives and amphotericin B. EP-A-0 433 679 also describes suitable Maillard inhibitors which include 4-hydroxy-5, 8-dioxoquinoline derivatives.

Active agents to be incorporated into a foamed glass using the methods of the invention are added to the mixture before the freezing step. A wide variety if substances can be incorporated. For example, bioactive substances such as pharmaceutical agents and biological modifiers can be processed according to the methods described herein.

Foam formation

A preferred process of the invention involves subjecting the preservation sample to such pressure and temperature conditions so that the sample begins to bubble, forming a foam.

The temperature within the preservation sample will be different from that external to the sample due to the endothermic nature of the evaporation process. References to temperature are to the conditions external to the preservation sample, for instance, where a large industrial freeze dryer is used, to the temperature of the shelf. This usually corresponds to the freeze dryer temperature setting.

A preferred embodiment of the invention causes foam formation to occur by changing the pressure while maintaining temperature conditions. The pressure is adjusted to at or below 8 , 7, 6, preferably 5, 4, 3, more preferably 2, 1.5, 1, most preferably 0.8 or 0.5 mbar while maintaining the temperature setting, corresponding to the temperature external to the preservation sample, at above 0 °C, preferably of between 10 °C to

15 °C; 15 °C to 20 °C; 20 °C to 25 °C; 25 °C to 30 °C; or 30 °C to 35 °C. These conditions are maintained for at least 1, 2, 3, 4, 5, 8, 10, 12, 16 or 24 hours.

Another embodiment of the invention achieves foam formation by increasing the temperature while maintaining or increasing the pressure conditions. The temperature setting is increased to above 20 °C, preferably to between 20 °C and 30 °C; 30 °C and 40 °C; 40 °C and 50 °C; or 50 °C and 70 °C; or the temperature setting is in the range of 10-50 °C, preferably 20-40 °C, more preferably 25-35 °C. Pressure conditions are set at or below at or below 8 , 7, 6, preferably 5, 4, 3, more preferably 2, 1.5, 1, most preferably 0.8, 0.5 0.2 or 0.1 mbar.

Removing solvent to form a solid

A subsequent stage of the method of the invention involves removing solvent until the foam dries to form a solid. In one embodiment of the invention, this is achieved by maintaining the pressure and temperature conditions at those applied in order to achieve foam formation. For instance, the pressure is maintained at or below 8 , 7, 6, preferably 5, 4, 3, more preferably 2, 1.5, 1, most preferably 0.8, 0.5 0.2 or 0.1 mbar while maintaining the temperature above 0 °C, preferably between 10 °C and 20 °C; 20 °C and 30 °C; 30 °C and 35 °C, most preferably between 5 °C and 25 °C. These temperature and pressure conditions are maintained for 1, 2, 3, 4, 5, 6, 8, 10, 12, 18 hours or more in order to obtain a solid with a solvent content less than or equal to15, 12, 10, 8, preferably 5, 4, 3, 2 or most preferably 1%.

Another embodiment of the invention increases the temperature setting during solvent removal to a higher temperature setting than that maintained earlier in the process. This allows the solvent to leave the sample at a quicker rate so that the method of the invention can be completed in a shorter time. For instance, the temperature setting is increased to above 0 °C, preferably between 10 °C and 20 °C; 20 °C and 30 °C; more preferably 30 °C and 40 °C; more preferably 40 °C and 50 °C; most preferably 50 °C and 60 °C while maintaining the pressure at or below 8 , 7, 6, preferably 5, 4,

3, more preferably 2, 1.5, 1, most preferably 0.8, 0.5 0.2 or 0.1 mbar. These temperature and pressure conditions are maintained for 1, 2, 3, 4, 5, 6, 8, 10, 12, 18 hours or more in order to obtain a solid with less than 15, 12, 10, 8, preferably 5, 4, 3, 2 or more preferably 1% water content.

Another embodiment of the invention reduces the pressure setting during solvent removal (step d) to a lower pressure setting than that used during foam formation (step c). This allows the solvent to leave the sample at a quicker rate so that the method of the invention can be completed in a shorter time. For instance, the pressure setting is set to at or below 7, 6, preferably 5, 4, 3, more preferably 2, 1.5, 1, most preferably 0.8, 0.5 0.2 or 0.1 mbar, while maintaining the temperature at or above 0 °C, preferably between 10 °C and 20 °C; 20 °C and 30 °C; 30 °C and 35 °C or above 40 °C. These temperature and pressure conditions are maintained for 1, 2, 3, 4, 5, 6, 8, 10, 12, 18 hours or more in order to obtain a solid with a solvent content less than or equal to 15, 12, 10, 8, preferably 5, 4, 3 or 2 or more preferably 1%.

In a preferred embodiment of the invention, the steps of freezing the sample within the freeze dryer and foam formation are performed at a constant temperature, preferably altering the pressure conditions.

In a further preferred embodiment the steps of freezing the sample within the freeze dryer, foam formation and solvent removal to form a solid, are performed at a constant temperature, preferably altering the pressure conditions.

In a further embodiment of the invention, both pressure and temperature conditions are different during the steps of freezing the sample, foam formation and solvent removal to form a solid.

Freezing step

The method of the invention may optionally involve freezing the sample, either wholly or partially. Freezing the sample prior to foam drying has the advantage of

increased reproducibility between of activity between samples foam dried in a batch due to foam drying commencing from the same physical conditions of the starting point for all samples.

Freezing may be carried out before subjected the sample to reduced pressure by placing the preservation sample at a temperature below 0 °C for a suitable amount of time to allow the sample to freeze either wholly or partially. Preferably the temperature used is at or below -10 °C, -15 °C, -20 °C, -30 °C, -40 °C, -70 °C or -140 °C. The sample may be left at a temperature below 0 °C for 1, 2, 3, 4, 5, 8, 16 or more hours to allow freezing to occur.

For some samples, particularly samples that are easily damaged by solvent crystal formation such as cell preparations or other biological systems , it is preferable to freeze the sample slowly at a rate of less than or equal to 0.1, 0.5, 1, 2, 3, 4, 5 °C per hour. Other compositions are preserved more effectively by freezing instantaneously, for instance by snap freezing in liquid nitrogen. This method may be particularly useful for proteins or viral particles. Freezing by evaporation also results in rapid freezing of the sample.

Alternatively, the preservation sample is frozen by subjecting the sample to reduced pressure so that evaporation of solvent leads to cooling. Such quench freezing is carried out within a bulk freeze dryer apparatus, at a shelf temperature of or above 0 °C, 10 °C, more preferably 15 °C, 20 °C, 30 °C or 37 °C. Preferably the shelf temperature is between 5 and 35°C, more preferably between 10 and 20 °C, most preferably at 15 °C. The pressure is optionally reduced initially to around 200mbar for 5, preferably 10, 20, 30, 60 minutes or more to allow degassing. In order to freeze the sample, the pressure is reduced further to a pressure equal to or below 2, 1, preferably 0.5, 0.2, most preferably 0.1mbar. This pressure is maintained for at least 5, 10, preferably 20 or 30 minutes until the sample is wholly or partially frozen.

The sample is subjected to temperature and pressure conditions suitable for allowing foam formation to occur and solvent is removed until a solid is formed as described above.

Active agent

Any substance that can be homogeneously suspended in a solution of a solvent and glass matrix-forming material can be processed using a method of the invention. Foamed glasses have a greatly increased surface area compared to the mixture, a solid dosage form or any previously described composition. The increased surface area allows facile dissolution and therefore this invention is applicable to a large number of substances. Determining whether a substance is suitable for use herein is within the skill of one in the art, and by the examples provided herein are illustrative and non-limiting. By foaming a homogeneous suspension, areas of unevenly distributed substance, which could be deleterious for dissolution, are avoided in foamed glass. More preferably, the substance will be solubilised in the solvent used in the initial mixture.

The active agent to be preserved using a method of the invention may comprise a biological system selected from the group consisting of cells, subcellular compositions, bacteria, outer membrane vesicle preparations and viruses, virus components or virus like particles. It may also comprise molecules, for instance proteins, peptides, amino acids, polynucleic acids, oligonucleotides, polysaccharides, oligosaccharides, polysaccharide – protein conjugates, oligosaccharide-protein conjugates.

Examples of active agents that can be preserved using a method of the invention include any bioactive substances such as pharmaceutically effective substances, including , but not limited to, antiinflammatory drugs, analgesics, tranquillisers, antianxiety drugs, antispasmodics, antidepressants, antipsychotics, tranquillisers, antianxiety drugs, narcotic antagonists, antiparkinsonism agents, cholinergic agonists, chemotherapeutic drugs, immunosuppressive agents, antiviral agents, antimicrobial

agents, appetite suppressants, anticholinergics, antimetrics, antihistaminics, antimigraine agents, coronary, cerebal or peropheral vasodilators, hormonal agents, contraceptives, antithrombotic agents, diueretics, antihypertensive agents, cardiovascular drugs, opioids, and the like.

Suitable agents also include therapeutic and prophylactic agents. These include, but are not limited to, any therapeutically effective biological modifier. Such substances include, but are not limited to, subcellular compositions, cells, bacteria, outer membrane vesicle preparations, viruses and molecules including but not limited to, lipids, organics, proteins and peptides (synthetic and natural), peptide mimetics, hormones (peptide, steroid and corticosteroid), D and L amino acid polymers, oligosaccharides, polysaccharides, nucleotides, oligonucleotides and nucleic acids, including DNA and RNA, protein nucleic acid hybrids, small molecules and physiologically active analogues thereof. Further, the modifiers may be derived from natural sources or made by recombinant or synthetic means and include analogues, agonists and homologs.

As used herein "protein" refers also to peptides and polypeptides. Such proteins include, but are not limited to, enzymes, biopharmaceuticals, growth hormones, growth factors, insulin, antibodies, both monoclonal and polyclonal and fragments thereof, interferons, interleukins and cytokines.

Therapeutic nucleic acid-based agents prepared by the methods described herein are also encompassed by the invention. As used herein, "nucleic acids" includes any therapeutically effective nucleic acids known in the art including, but not limited to DNA, RNA, and physiologically active analogues thereof. The nucleotides may encode genes or may be any vector known in the art of recombinant DNA including, but not limited to, plasmids, retroviruses and adeno-associated viruses.

The preservation of substances which are prophylactically active and carriers thereof are further encompassed by the invention. Preferable compositions include immunogens such as vaccines. Vaccines may be for oral administration or may be for

injection after reconstitution. Suitable vaccines include, but are not limited to, live and attenuated viruses, nucleotide vectors encoding antigens, live and attenuated bacteria, protein, polysaccharide, oligosaccharide and/or lipopolysaccharide antigens, antigens plus adjuvants and antigens and/or haptens coupled to carriers. Particularly preferred are vaccines effective against diptheria, tetanus, pertussis, botulinum, cholera, Dengue, Hepatitis A, B, C and E, *Haemophilus influenzae* b, *Streptococcus pneumoniae, Neisseria meningitidis, Neisseria gonorrhoeae, Staphylococcus aureus, Staphylococcus epidermidis*, Group B streptococci, Group A streptococci, herpes virus, *Helicobacterium pylori*, influenza, Japanese encephalitis, meningococci A, B, C, Y, W, measles, mumps, papilloma virus, pneumococci, polio virus, inactivated polio virus (IPV – preferably comprising types 1, 2 and 3 as is standard in the vaccine art, most preferably the Salk polio vaccine), rubella, rotavirus, respiratory syncytial virus, Shigella, tuberculosis, yellow fever and combinations thereof. The antigenic component of vaccines may also be produced by molecular biology techniques to produce recombinant peptides or fusion proteins containing one or more portions of a protein derived from a pathogen. For instance, fusion proteins containing an antigen and the B subunit of cholera toxin have been shown to induce an immune response to the antigen. Sanches et al (1989) Proc. Natl. Acad. Sci. USA 86:481-485. Vaccines are particularly suitable for incorporation into the single-dosage composition. They are stable indefinitely under ambient conditions and can be redissolved in sterile diluent immediately before inoculation.

Combinations of two or more of the above active agents may be preserved using the method of preservation of the invention. Part or all of a vaccine may be preserved using the method of preservation of the invention.

Preferred combinations of active agents to be preserved using the process of the invention comprise IPV (an inactivated mixture of polio virus strains).

IPV is defined as inactivated polio virus (preferably comprising types 1, 2 and 3 as is standard in the vaccine art, most preferably the Salk polio vaccine). A vaccine dose of IPV contains 20-80, preferably 40 or 80 D-antigen units of type 1 (Mahoney), 4-

16, preferably 8 or 16 D-antigen units of type 2 (MEF-1) and 20-64, preferably 32 or 64 D-antigen units of type 3 (Saukett).

Preferably, the drying process utilised retains the IPV antigens so that an ELISA on the reconstituted dried composition , using antibodies against polio virus type 1, 2 and/or 3, gives results that are over 40%, 50%, preferably 60%, 70%, more preferably 80% or 90% the level achieved using the undried IPV. In vivo experiments in which the dried formulation, after reconstitution is inoculated into an animal, preferably a mouse may also be used to assess the degree of antigen retention.

Preferably, IPV is combined with one or more of Hib (*Haemophilus influenzae* type b) PRP polysaccharide and/or meningococcal A, C, W and/or Y polysaccharides and/or pneumococcal polysaccharides. Most preferably the active agents comprise, IPV and Hib; IPV and MenC; IPV, Hib and MenC; Hib and MenC; IPV and MenA and C; Hib and Men A and C; IPV, Hib, Men A and C; Hib, Men C and Y; or IPV, Hib, Men C and Y.

The above particularised active agents may also comprise one or more pneumococcal capsular polysaccharides as described below.

In the above compositions where polysaccharides are used, oligosaccharides may also be employed (as defined below).

Although these compositions may be adjuvanted (as described below), they are preferably unadjuvanted or preferably do not comprise aluminium salts.

Preferably the polysaccharides or alogosaccharides are conjugated to a peptide or carrier protein comprising T-helper epitopes (as described below).

Additional components

The preferred combinations, foam dried by the process of the invention may be combined with other antigens in a combination vaccine which is desiccated or is preferably a liquid formulation which can be used to reconstitute the foam dried components. Preferred antigens to be combined with the active agents in the paragraph above include one or more of diphtheria toxoid, tetanus toxoid, whole cell pertussis (Pw), acellular pertussis (Pa) (as described below), Hepatitis B surface antigen, pneumococcal polysaccharides, pneumococcal proteins or any of the antigens listed below. Bacterial polysaccharides may be conjugated to a carrier protein such as tetanus toxoid, tetanus toxoid fragment C, diphtheria toxoid, CRM197, pneumolysin, Protein D (US6342224) as described below.

Active agents preserved using the process of the invention may be formulated with capsular polysaccharides derived from one or more of *Neisseria meningitidis*, *Haemophilus influenzae* b, *Streptococcus pneumoniae*, Group A Streptococci, Group B Streptococci, *Staphylococcus aureus* or *Staphylococcus epidermidis*. In a preferred embodiment, the immunogenic composition would comprise capsular polysaccharides derived from one or more of serogroups A, C, W-135 and Y of *Neisseria meningitidis*. A further preferred embodiment would comprise capsular polysaccharides derived from *Streptococcus pneumoniae*. The pneumococcal capsular polysaccharide antigens are preferably selected from serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F (most preferably from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F). A further preferred embodiment would contain the PRP capsular polysaccharides of *Haemophilus influenzae* type b. A further preferred embodiment would contain the Type 5, Type 8 or 336 capsular polysaccharides of *Staphylococcus aureus*. A further preferred embodiment would contain the Type I, Type II or Type III capsular polysaccharides of *Staphylococcus epidermidis*. A further preferred embodiment would contain the Type Ia, Type Ic, Type II or Type III capsular polysaccharides of Group B streptocoocus. A further preferred embodiment would contain the capsular polysaccharides of Group A streptococcus, preferably further comprising at least one M protein and more preferably multiple types of M protein.

In one embodiment of the invention, the bacterial polysaccharides are full length, being purified native polysaccharides. In an alternative embodiment of the invention, the polysaccharides are sized between 2 and 20 times, preferably 2-5 times, 5-10 times, 10-15 times or 15-20 times, so that the polysaccharides are smaller in size for greater manageability. Oligosaccharides are used in a preferred embodiment. Oligosaccharides typically contain between 2 and 20 repeat units.

Preferred pneumococcal proteins antigens are those pneumococcal proteins which are exposed on the outer surface of the pneumococcus (capable of being recognised by a host's immune system during at least part of the life cycle of the pneumococcus), or are proteins which are secreted or released by the pneumococcus. Most preferably, the protein is a toxin, adhesin, 2-component signal tranducer, or lipoprotein of *Streptococcus pneumoniae*, or fragments thereof. Particularly preferred proteins include, but are not limited to: pneumolysin (preferably detoxified by chemical treatment or mutation) [Mitchell *et al.* Nucleic Acids Res. 1990 Jul 11; 18(13): 4010 "Comparison of pneumolysin genes and proteins from *Streptococcus pneumoniae* types 1 and 2.", Mitchell *et al.* Biochim Biophys Acta 1989 Jan 23; 1007(1): 67-72 "Expression of the pneumolysin gene in *Escherichia coli*: rapid purification and biological properties.", WO 96/05859 (A. Cyanamid), WO 90/06951 (Paton et al), WO 99/03884 (NAVA)]; PspA and transmembrane deletion variants thereof (US 5804193 - Briles *et al.*); PspC and transmembrane deletion variants thereof (WO 97/09994 - Briles et al); PsaA and transmembrane deletion variants thereof (Berry & Paton, Infect Immun 1996 Dec;64(12):5255-62 "Sequence heterogeneity of PsaA, a 37-kilodalton putative adhesin essential for virulence of *Streptococcus pneumoniae*"); pneumococcal choline binding proteins and transmembrane deletion variants thereof; CbpA and transmembrane deletion variants thereof (WO 97/41151; WO 99/51266); Glyceraldehyde–3-phosphate – dehydrogenase (Infect. Immun. 1996 64:3544); HSP70 (WO 96/40928); PcpA (Sanchez-Beato et al. *FEMS Microbiol Lett* 1998, 164:207-14); M like protein, (EP 0837130) and adhesin 18627, (EP 0834568). Further preferred pneumococcal protein antigens are those disclosed in WO 98/18931, particularly those selected in WO 98/18930 and PCT/US99/30390.

Preferred Neisserial proteins to be formulated with the immunogenic composition of the invention include TbpA (WO93/06861; EP586266; WO92/03467; US5912336), TbpB (WO93/06861; EP586266), Hsf (WO99/31132), NspA (WO96/29412), Hap (PCT/EP99/02766), PorA, PorB, OMP85 (also known as D15) (WO00/23595), PilQ (PCT/EP99/03603), PldA (PCT/EP99/06718), FrpB (WO96/31618 see SEQ ID NO:38), FrpA or FrpC or a conserved portion in common to both of at least 30, 50, 100, 500, 750 amino acids (WO92/01460), LbpA and/or LbpB (PCT/EP98/05117; Schryvers et al Med. Microbiol. 1999 32: 1117), FhaB (WO98/02547), HasR (PCT/EP99/05989), lipo02 (PCT/EP99/08315), MltA (WO99/57280) and ctrA (PCT/EP00/00135).

The vaccine may also optionally comprise antigens providing protection against one or more of Diphtheria, tetanus and *Bordetella pertussis* infections. The pertussis component may be killed whole cell *B. pertussis* (Pw) or acellular pertussis (Pa) which contains at least one antigen (preferably two or all three) from PT, FHA and 69kDa pertactin. Typically, the antigens providing protection against Diphtheria and tetanus would be Diphtheria toxoid and tetanus toxoid. The toxoids may chemically inactivated toxins or toxins inactivated by the introduction of point mutations.
Alternatively the foamed glass of the invention may be provided as a kit with the foamed glass in one container and liquid DTPa or DTPw in another container. The foamed glass is reconstituted with the liquid DTPa or DTPw vaccine (preferably extemporaneously) and administered as a single vaccine. The DTPa or DTPw vaccine typically is adjuvanted at least in part with aluminium hydroxide (for instance Infanrix ® and Tritanrix ® vaccines of GlaxoSmithKline Biologicals s.a.).

The vaccine may also optionally comprise one or more antigens that can protect a host against non-typeable *Haemophilus influenzae*, RSV and/or one or more antigens that can protect a host against influenza virus.

Preferred non-typeable *H. influenzae* protein antigens include Fimbrin protein (US 5766608) and fusions comprising peptides therefrom (eg LB1 Fusion) (US 5843464 -

Ohio State Research Foundation), OMP26, P6, protein D, TbpA, TbpB, Hia, Hmw1, Hmw2, Hap, and D15.

Preferred influenza virus antigens include whole, live or inactivated virus, split influenza virus, grown in eggs or MDCK cells, or Vero cells or whole flu virosomes (as described by R. Gluck, Vaccine, 1992, 10, 915-920) or purified or recombinant proteins thereof, such as HA, NP, NA, or M proteins, or combinations thereof.

Preferred RSV (Respiratory Syncytial Virus) antigens include the F glycoprotein, the G glycoprotein, the HN protein, the M protein or derivatives thereof.

It should be appreciated that antigenic compositions of the invention may comprise one or more capsular polysaccharide from a single species of bacteria. Antigenic compositions may also comprise capsular polysaccharides derived from one or more species of bacteria.

Such capsular polysaccharides may be unconjugated or conjugated to a carrier protein such as tetanus toxoid, tetanus toxoid fragment C, diphtheria toxoid, CRM197, pneumolysin, Protein D (US6342224). Tetanus toxin, diphtheria toxin and pneumolysin are detoxified either by genetic mutation and/or preferably by chemical treatment.

The polysaccharide conjugate may be prepared by any known coupling technique. For example the polysaccharide can be coupled via a thioether linkage. This conjugation method relies on activation of the polysaccharide with 1-cyano-4-dimethylamino pyridinium tetrafluoroborate (CDAP) to form a cyanate ester. The activated polysaccharide may thus be coupled directly or via a spacer group to an amino group on the carrier protein. Preferably, the cyanate ester is coupled with hexane diamine and the amino-derivatised polysaccharide is conjugated to the carrier protein using heteroligation chemistry involving the formation of the thioether linkage. Such conjugates are described in PCT published application WO93/15760 Uniformed Services University.

The conjugates can also be prepared by direct reductive amination methods as described in US 4365170 (Jennings) and US 4673574 (Anderson). Other methods are described in EP-0-161-188, EP-208375 and EP-0-477508.

A further method involves the coupling of a cyanogen bromide activated polysaccharide derivatised with adipic acid hydrazide (ADH) to the protein carrier by Carbodiimide condensation (Chu C. et al Infect. Immunity, 1983 245 256).

Preferably, the immunogenic composition or vaccine contains an amount of an adjuvant sufficient to enhance the immune response to the immunogen. Suitable adjuvants include, but are not limited to, aluminium salts, squalene mixtures (SAF-1), muramyl peptide, saponin derivatives, mycobacterium cell wall preparations, monophosphoryl lipid A, mycolic acid derivatives, non-ionic block copolymer surfactants, Quil A, cholera toxin B subunit, polphosphazene and derivatives, and immunostimulating complexes (ISCOMs) such as those described by Takahashi et al. (1990) Nature 344:873-875. For veterinary use and for production of antibodies in animals, mitogenic components of Freund's adjuvant can be used.

As with all immunogenic compositions or vaccines, the immunologically effective amounts of the immunogens must be determined empirically. Factors to be considered include the immunogenicity, whether or not the immunogen will be complexed with or covalently attached to an adjuvant or carrier protein or other carrier, route of administrations and the number of immunising dosages to be administered. Such factors are known in the vaccine art and it is well within the skill of immunologists to make such determinations without undue experimentation.

The active agent can be present in varying concentrations in the foamed glass of the invention. Typically, the minimum concentration of the substance is an amount necessary to achieve its intended use, while the maximum concentration is the maximum amount that will remain in solution or homogeneously suspended within the initial mixture. For instance, the minimum amount of a therapeutic agent is preferably one which will provide a single therapeutically effective dosage. Super-

saturated solutions can also be used if the foamed glass is formed prior to crystallisation. For bioactive substances, the minimum concentration is an amount necessary for bioactivity upon reconstitution and the maximum concentration is at the point at which a homogeneous suspension cannot be maintained. In the case of single-dosed units, the amount is that of a single therapeutic application. Generally, it is expected that each dose will comprise 1-100ug of protein antigen, preferably 5-50ug and most preferably 5-25ug. Preferred doses of bacterial polysaccharides are 10-20ug, 10-5ug, 5-2.5ug or 2.5-1ug. The preferred amount of the substance varies from substance to substance but is easily determinable by one of skill in the art.

Foamed glass comprising an active agent

Another aspect of the invention is a foamed glass comprising an active agent which is obtainable or obtained using a method of the invention. Foamed glasses of the invention may contain any of the active agents described above. The active agent preserved by the foamed glass may comprise a biological system, for instance cells, subcellular compositions, bacteria, outer membrane vesicle preparations and viruses. It may alternatively or further comprise a molecules, for example proteins, peptides, amino acids, polynucleic acids, oligonucleotides, polysaccharides, oligosaccharides, polysaccharide – protein conjugates, oligosaccharide-protein conjugates. It may also comprise combinations of comprising two or more of the above active agents.

Preferred embodiments include a foamed glass obtained or obtainable by a method of the invention wherein the active agent is or comprises a vaccine. Preferred components of the vaccine are described above and include IPV and bacterial polysaccharides from *Haemophilus influenzae* b and *Neisseria meningitidis* A, C, W and Y.

A container with a water repellent interior surface holding a foamed glass

A further aspect of the invention is a container with a water repellent interior surface containing a foamed glass comprising an active agent. The use of such a container to

prepare a foamed glass, preserving an active agent will have advantages in terms of ease of foam formation, a reduction in the amount of stabilising agent required and a reduction in the time to carry out the foam drying of the sample.

In the case of vaccines to be reconstituted contemporaneously with inoculation, further advantages include ease of reconstitution and ease of manipulation and injection.

The container with a water repellent interior surface, containing a foamed glass is made using a process of the invention as described above. The container of the invention will therefore contain a glass forming polyol, for instance glucose, maltulose, iso-maltulose, lactulose, sucrose, sorbitol, maltose, lactose, iso-maltose, maltitol, lactitol, palatinit, trehalose, raffinose, stachyose, melezitose or dextran. Additionally it preferably contains effervescent salts

The container of the invention preferably comprises an active agent such as protein, peptide, amino acid, polynucleotide, oligonucleotide, polysaccharide, oligosaccharide, polysaccharide-protein conjugate and oligosaccharide-protein conjugate or a biological system such as cells, subcellular compositions, bacteria, outer membrane vesicle preparations, viruses, virus components and virus like particles. Preferred active agents are described above.

Preferably, the container of the invention comprises a vaccine. Preferred vaccine components include IPV (an inactivated mixture of polio virus strains). Preferably, IPV is combined with one or more of Hib PRP polysaccharide and/or meningococcal A, C, W and/or Y polysaccharides and/or pneumococcal polysaccharides (as described above), more preferably IPV and Hib; IPV and MenC; IPV, Hib and MenC; Hib and MenC; IPV and MenA and C; Hib and Men A and C; IPV, Hib, Men A and C; Hib, Men C and Y; or IPV, Hib, Men C and Y.

The contents of the container of the invention are preferably combined with other antigens in a combination vaccine which are optionally desiccated or preferably liquid

formulations which can be used to reconstitute the foam dried components. Preferred antigens to be combined with the contents of the container of the invention include one or more of diphtheria toxoid, tetanus toxoid, whole cell pertussis (Pw), acellular pertussis (Pa) (as described above), Hepatitis B surface antigen, pneumococcal polysaccharides, pneumococcal proteins, neisserial polysaccharides, neisserial proteins. Bacterial polysaccharides may be conjugated to a carrier protein such as tetanus toxoid, tetanus toxoid fragment C, diphtheria toxoid, CRM197, pneumolysin, Protein D (US6342224) as described above.

All references or patent applications cited within this patent specification are incorporated by reference herein.

Examples

The examples below are carried our using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples are illustrative, but do not limit the invention.

Example 1. Evaporative freezing process

The process was carried out using a Heto Drywinner 8-85 freeze-dryer in which shelf temperature may be regulated to within 1 °C , the final temperature of the condenser is -85 °C , pressure is regulated with a bleed valve and 6 thermocouples are available to measure the product temperature.

A preservation sample was made by adding a polyol and an active agent to an aqueous solution. Samples were put into the freeze dryer with a shelf temperature maintained at 15 °C throughout the process. The pressure was initially reduced to 200mBar and maintained at this level for 10 minutes before reducing the pressure further. At 1.5mBar, the solutions begin to freeze due to evaporative cooling as shown in figure 1. The pressure is further reduced to 0.1mBar to allow the samples to become fully frozen. The pressure was then increased to between 0.8mBar and 3.5mBar at which point a foam formed as water was lost from the sample. Under the conditions of the experiment, no boiling was seen in a control sample containing only water. The samples may be loosing water through evaporation rather than through boiling. After 18 hours under these conditions, the samples are dried and the foamed solution becomes a foamed glass.

Similar processes were performed keeping the shelf temperature at other temperature settings up to 37 °C.

Example 2. Establishment of freezing conditions

Samples were made by dissolving sucrose in water to give1%, 5%, 10% and 20% solutions. Samples were put into the freeze dryer with a shelf temperature maintained at 15 °C throughout the process. The pressure was initially reduced to 200mBar and maintained at this level for 10 minutes before reducing the pressure further to 50mBars, 5mBars, 2.5mBars, 0.75mBars, 0.4mBars and 0.2mBars. Each pressure level was maintained for 20 minutes to allow the temperature to equilibrate and the temperature of the sample was read using a thermocouple. Thermocouples were attached to samples with different sucrose concentrations and the temperatures recorded in table 1 are mean values of the temperatures.

Results

All samples froze between 1.66 and 1.11mbars, irrespective of the concentration of sucrose present. The temperatures measured at different pressures were very close to those predicted from the triple point curve. Therefore the presence of sucrose does not have a large effect on the temperature of the samples at different pressures.

Table 1

Pressure	Measured temperature	Theoretical temperature	Liquid/frozen
1000mBar	15 °C		liquid
50mBar	15 °C		liquid
5mBar	1 °C	1 °C	liquid
2.5mBar	-5 °C	-7 °C	liquid
0.75mBar	-21 °C	-21 °C	frozen
0.4mBar	-22 °C	-27 °C	frozen
0.2mBar	-27 °C	-32 °C	frozen

Example 3. Foaming conditions for samples with different sugar concentrations

Preservation samples containing 0%, 5%, 10%, 15%, 20%, 25% and 50% sucrose were made. Samples were put into the freeze dryer with a shelf temperature maintained at 15 °C throughout the process. The pressure was initially reduced to 200mbars and maintained at this level for 10 minutes before reducing the pressure further. The pressure was further reduced to 0.1mbars to allow the samples to become fully frozen. The pressure was then increased to either 0.788mbars, 0.812mbars or 3.5mbars in subsequent experiment These conditions were maintained for 3 hours for the 3.5mbars and 0.812mbars experiments and for 6 hours for the 0.788 mbars experiment. The physical characteristics of each sample were evaluated.

Results

As shown in table 2, at a pressure of 3.5mbars, a high sucrose concentration of 50% was required for reliable formation of foam. In contrast, a lower pressure of 0.8mbars allowed reliable foam formation at lower sucrose concentrations of 10-25%. The use of lower sucrose concentration could be advantageous for preserved samples to be used in vaccines for instance. Therefore a process using 0.8mbars and a low sucrose content is preferred.

Table 2

Pressure	%sucrose	Physical characteristics
3.5mbars	20	4/5 foamed, 1/5 viscous liquid
3.5mbars	25	2/5 foamed, 3/5 viscous liquid
3.5mbars	50	5/5 foamed
0.812mbars	5	Ring of crystallisation and bubbles
0.812mbars	10	All foamed
0.812mbars	15	All foamed
0.812mbars	20	All foamed
0.812mbars	25	All foamed
0.788mbars	5	Ring of crystallisation and bubbles
0.788mbars	20	All foamed
0.788mbars	25	All foamed
0.788mbars	50	Foam and syrup

Example 4. The effect of using siliconized containers

Preservation samples containing 5%, 10%, 15% and 25% sucrose were made and added to vials, some of which were siliconized. In one experiment, samples were put into the freeze dryer with a shelf temperature maintained at 15 °C throughout the process. The pressure was initially reduced to 200mbars and maintained at this level for 10 minutes before reducing the pressure further. The pressure was further reduced to 2.8mbars for 3 hours. During this period, the pressure fell to 2.00mbars as the presence of water vapour decreased. The physical characteristics of each sample were evaluated.

In a second experiment, samples were put into the freeze dryer with a shelf temperature maintained at 37°C throughout the process. The pressure was initially reduced to 200mbars and maintained at this level for 10 minutes before reducing the pressure further. The pressure was further reduced to 2.4mbars for 3 hours.During this

period, the pressure fell to 1.06mbars as the presence of water vapour decreased. The physical characteristics of each sample were evaluated.

Results

Siliconization had an effect on the degassing of the samples. The reduction of pressure to 200mbars resulted in degassing of samples in siliconized vials but not in unsiliconized vials. Degassing was seen by bubbling of the sample.

The siliconisation of the vial also made foam formation more likely to occur and more reproducible (table 3). Siliconisation of vials allows foam formation to occur reproducibly at lower polyol concentrations. The lower polyol concentration decreases the length of time necessary to dry the sample and reduces the effect of Maillard reactions or other interactions with the polyol harming the active agent. Where the sample involved is a vaccine, this reduces the viscosity of the sample and allows easier administration.

Table 3

Temperature and pressure	% sucrose	Characteristics nonsiliconised vial	Characteristics siliconised vial
15°C, 2.8mbars	5%	Viscous fluid	
15°C, 2.8mbars	10%	Viscous fluid	foamed
15°C, 2.8mbars	15%	Viscous fluid	
15°C, 2.8mbars	25%	Viscous fluid	
37°C, 2.4mbars	5%	3 viscous fluid 2 foamed	
37°C, 2.4mbars	10%	All viscous fluid	5 foamed 1 viscous fluid
37°C, 2.4mbars	15%	All foamed	
37°C, 2.4mbars	25%	All foamed	

Example 5. Comparison of preservation of Hib-IPV by conventional freeze drying or by foam drying

The active agent to be preserved was a mixture of the PRP polysaccharide of Haemophilus influenzae b (Hib) and three strains of inactivated polio virus (IPV). The preservation sample was made by dissolving Hib-IPV in either a 3.15% sucrose solution or a 10% trehalose solution.

The samples were lyophilised either by using a conventional freeze drying sample that required three days to perform in a large freeze dryer, or by using the foam drying method described in example 1.

The samples were reconstituted in water and an ELISA was used to assess the integrity of structure of the three polio virus strains. Three polyclonal antibodies and three monoclonals, one against each strain, were used in separate ELISAs. Results are

presented as a percentage of the reading given for a sample which had not undergone the freeze drying or foam drying procedure.

The preserved samples are assessed for their immunogenicity in vivo by inoculating groups of ten mice with the reconstituted IPV-Hib, withdrawing blood from the mice and monitoring levels of antibodies against IPV and Hib polysaccharides, for instance by ELISA or Western blotting. The degree of protection is assessed in a challenge mouse model.

Results

Using either sucrose or trehalose as the polyol, the integrity of IPV was maintained better using the foam drying technique compared to using conventional freeze drying (table 4).

Table 4

Method of drying	Polyol content	ELISA – type 1/2/3 %	
		Polyclonal	Monoclonal
Freeze drying	3.15% sucrose	46/49/58*	25/0/0
Foam drying	3.15% sucrose	85/97/106	55/68/57
Freeze drying	10% trehalose	47/43/58	
Foam drying	10% trehalose	93/86/84	72/75/87

* The experiment freeze drying in the presence of 3.15% sucrose was repeated five times and the results shown are from one representative experiment.

Example 6. Reproducibility of sample quality after freeze drying, foam drying or foam drying with a freezing step.

Preservation samples are made up comprising IPV, mumps, measles, rubella, varicella zoster virus, CMV, hepatitis, HSV1, HSV2, respiratory syncitial virus, dengue, paramyxoviridae such as parainfluenza, togaviridae and influenza viruses, and/or Hib as the active agent. The active agent are dissolved in an aqueous solution containing a polyol. Multiple samples are preserved by either freeze drying, foam drying using a freezing step following the protocol described in example 1, or foam drying without a freezing step using a protocol described in example 4. Samples are reconstituted in an aqueous solution and their activity assessed. This is accomplished using ELISA assays as described in example 5 using antibodies specific to native antigens. In the case of live viruses, the titre of each sample is established by using the virus to infect suitable host cells and assessing the infectivity by plaque formation or by immunocytochemistry. Where immunogenic compositions or vaccines are foam dried, the integrity is tested in an animal model by immunising groups of animals with vaccine which is foam dried or freeze dried and boosting the immune response for instance at 14 and 28 days after the first immunisation. Serum is isolated from animals at the end of the immunisation schedule and its titre against the vaccine is

tested using standard assays, for instance by ELISA, immunocytochemistry, Western blotting, immunoprecipitation, serum bacteriocidal assay or agglutination assay. Results are complied, firstly by comparing the activity of the active agent after freeze drying, foam drying with a freezing step, or foam drying without a freezing step. Secondly, the degree of reproducibility of the preservation technique is assessed by comparing the range of activities after subjecting samples to each of the three preservation methods.

Example 7. Long term storage of active agents preserved by freeze drying, and foam drying.

Preservation samples are made up comprising IPV, mumps, measles, rubella, varicella zoster virus, CMV, hepatitis, HSV1, HSV2, respiratory syncitial virus, dengue, paramyxoviridae such as parainfluenza, togaviridae and influenza viruses, and/or Hib as the active agent. The active agent is dissolved in an aqueous solution containing a polyol. Multiple samples are preserved by either freeze drying, foam drying using a freezing step following the protocol described in example 1, or foam drying without a freezing step using a protocol described in example 4. Samples are aged by storing at 37°C or 23°C for seven days and are compared for activity with samples that have been keep at 4°C . Samples are reconstituted in an aqueous solution and their activity assessed. This is accomplished using ELISA assays as described in example 5 using antibodies specific to native antigens. In the case of live viruses, the titre of each sample is established by using the virus to infect suitable host cells and assessing the infectivity by plaque formation or by immunocytochemistry. Results are complied, firstly by comparing the activity of the active agent after storage at elevated temperatures with storage at 4°C. Secondly, the degree of reproducibility of the preservation technique is assessed by comparing the range of activities after subjecting samples to each set of conditions.

Claims

1. A method for preserving an active agent comprising the steps of:
a) preparing a preservation sample by dissolving/suspending an active agent in a solution of a stabilising agent;
b) inserting the preservation sample into a container with a water repellent interior surface;
c) subjecting the container holding the preservation sample to such temperature and pressure conditions so that the preservation sample forms a foam;
d) removing solvent until the foam dries to form a solid.

2. The method of claim 1 wherein the pressure is reduced to 8mbars or below.

3. The method of claim 1 wherein the pressure is reduced to 4mbars or below.

4. The method of claim 1 wherein the pressure is reduced to 1mbar or below.

5. The method of claim 1 wherein the pressure is reduced to 0.2mbars or below.

6. The method of claim 1-5 wherein the temperature external to the preservation sample is constant during steps c) and d).

7. The method of claim 6 wherein the temperature external to the preservation sample is between 5°C and 25°C.

8. The method of claim 1-5 wherein the temperature external to the preservation sample is higher during step d) than it is in step c).

9. The method of claim 1-5 wherein the pressure is reduced in step c) compared to the pressure during step b).

10. The method of claim 1-9 wherein the preservation sample becomes at least partially frozen.

11. The method of claims 1-10 wherein the stabilising agent comprises a glass forming polyol, selected from the group consisting of glucose, maltulose, iso-maltulose, lactulose, sucrose, maltose, lactose, sorbitol, iso-maltose, maltitol, lactitol, palatinit, trehalose, raffinose, stachyose, melezitose and dextran.

12. The method of claim 11 wherein the concentration of stabilising agent is less than 15%.

13. The method of claims 1-12 wherein the active agent comprises a molecule selected from the group consisting of protein, peptide, amino acid, polynucleotide, oligonucleotide, polysaccharide, oligosaccharide, polysaccharide-protein conjugate and oligosaccharide-protein conjugate.

14. The method of claim 1-12 wherein the active agent comprises a biological system selected from the group consisting of cells, subcellular compositions, bacteria, viruses, virus components and virus like particles.

15. The method of claim 14 wherein the active agent comprises IPV (inactivated polio virus).

16. The method of claim 14-15 wherein the active agent comprises Hib (*Haemophilus influenzae* tyep b)polysaccharide or oligosaccharide.

17. The method of claim 14-16 wherein the active agent comprises *Neisseria meningitidis* C polysaccharide or oligosaccharide.

18. The method of claims 1-17 wherein the active agent comprises a vaccine.

19. A container with a water repellent interior surface, holding a foamed glass comprising an active agent, preferably obtainable by the method of claim 1-18.

20. The container of claim 19 wherein the foamed glass comprises a polyol selected from the group consisting of glucose, maltulose, iso-maltulose, lactulose, sucrose, sorbitol, maltose, lactose, iso-maltose, maltitol, lactitol, palatinit, trehalose, raffinose, stachyose, melezitose and dextran.

21. The container of claim 19-20 wherein the active agent comprises a molecule selected from the group consisting of protein, peptide, amino acid, polynucleotide, oligonucleotide, polysaccharide, oligosaccharide, polysaccharide-protein conjugate and oligosaccharide-protein conjugate.

22. The container of claim 19-21 wherein the active agent comprises a biological system selected from the group consisting of cells, subcellular compositions, bacteria, viruses, virus components and virus like particles.

23. The container of claim 19-22 wherein the active agent comprises a vaccine.

24. The container of claim 19-23 wherein the foamed glass comprises IPV.

25. The container of claim 19-24 wherein the foamed glass comprises IPV and a bacterial polysaccharide.

26. The container of claim 19-25 wherein the foamed glass comprises Hib (*Haemophilus influenzae* b) polysaccharide or oligosaccharide, preferably conjugated to a carrier protein.

27. The container of claim 19-26 wherein the foamed glass comprises Neisseria meningitidis serogroup C polysaccharide or oligosaccharide, preferably conjugated to a carrier protein.

28. A method of making a vaccine comprising the step of reconstituting the foamed glass in the container of claims 19-28 in an aqueous solution.

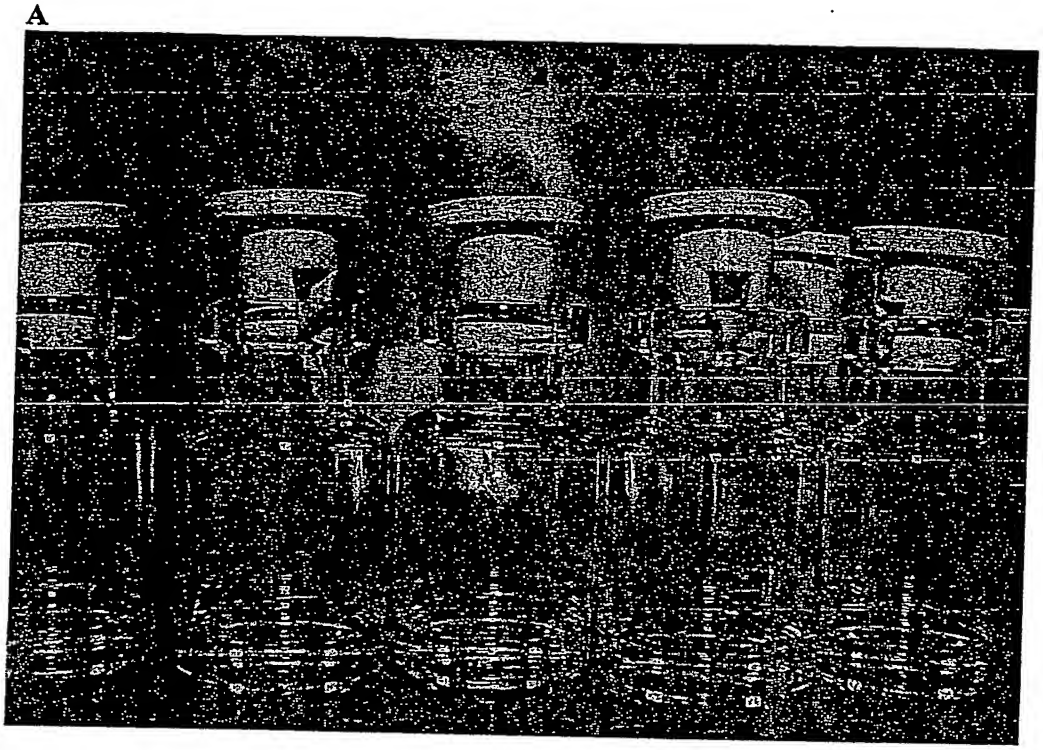
29. The method of claim 38 wherein the aqueous solution comprises D, T and P (acellular or whole cell) vaccine.

30. The method of claim 39 where the DTP vaccine is at least in part adjuvanted with aluminium hydroxide.

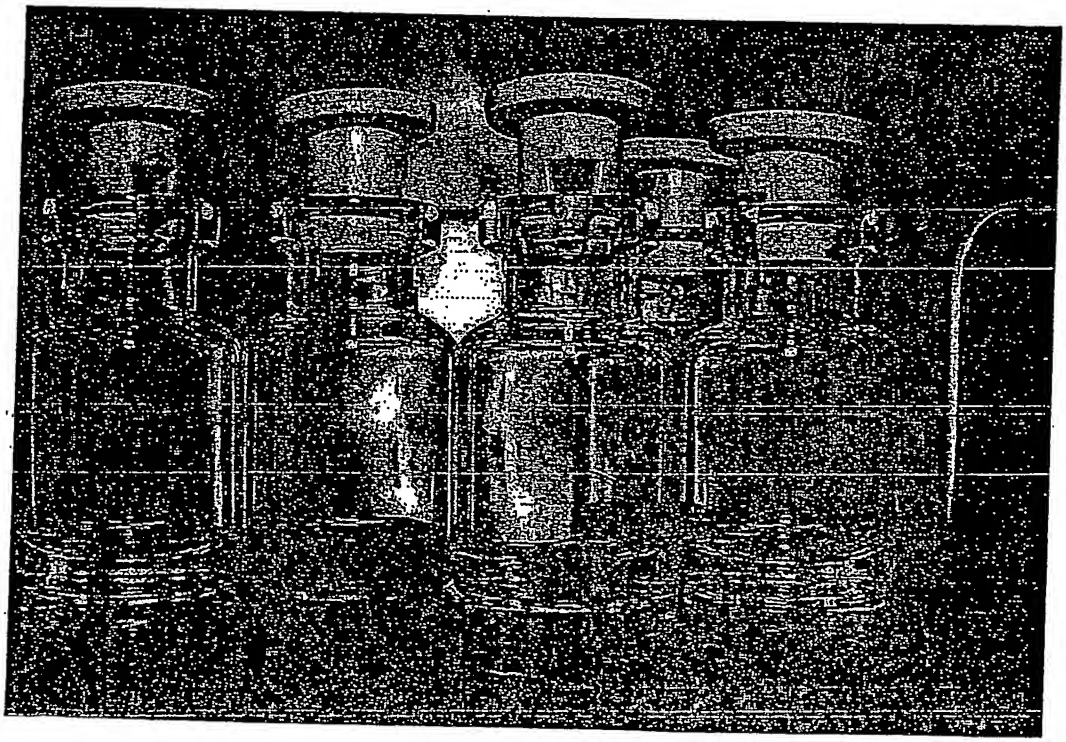
31. A kit comprising the container of claims 19-28 and liquid DTP (acellular or whole cell) vaccine in a second container.

Figure 1

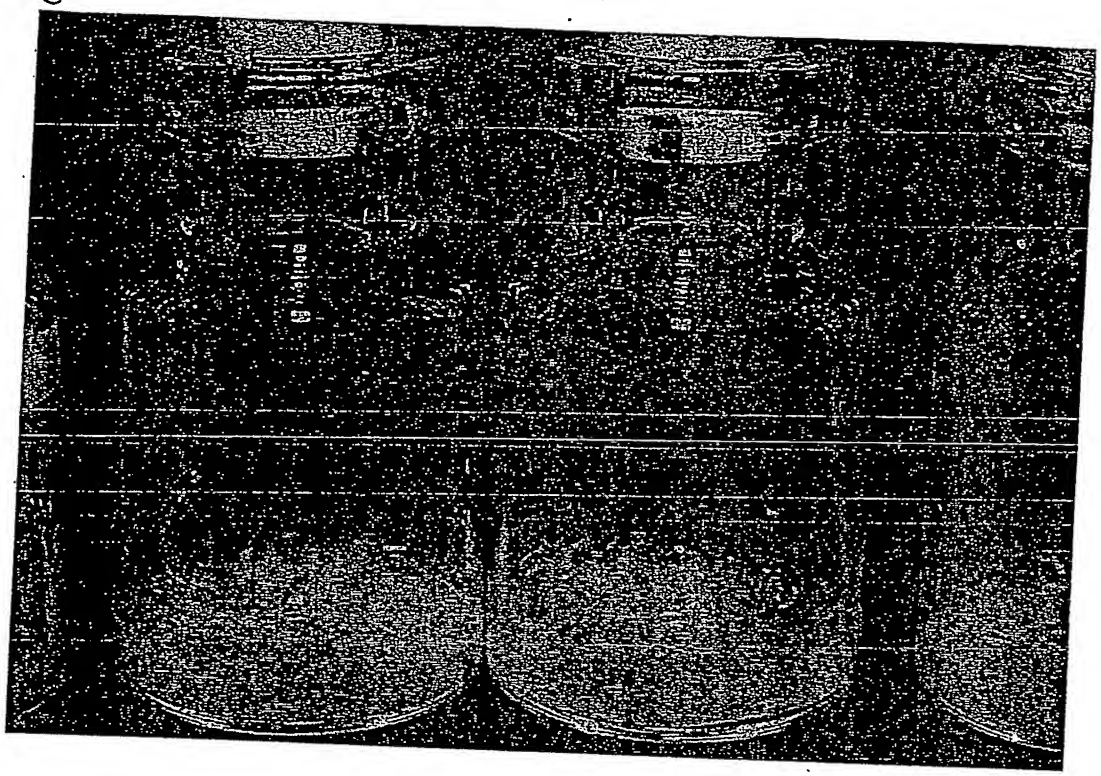
A



B



C



D